

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

KEITH CHADWICK MURDOCK and
FREDERICK EMIL DURR

Serial No. 63,285
Filed August 2, 1979

1,4-BIS(SUBSTITUTED-AMINO)-5,8-
-DIHYDROXY-ANTHRAQUINONES AND
LEUCO BASES THEREOF

Hon. Commissioner of Patents
and Trademarks
Washington, D. C. 20231

S i r:

AFFIDAVIT UNDER RULE 132

STATE OF NEW YORK)
) ss.
COUNTY OF ROCKLAND)

ROSLYN E. WALLACE, residing at 27 North Pearl Street, Pearl River,
New York 10965, being duly sworn, deposes and says;

THAT she is a trained Biologist, having received the Bachelor of Arts degree in Biology-Chemistry from Brenau College, Gainesville, Georgia in 1944;

THAT she has been employed since 1950 by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York as a Biologist;

THAT she has read and is familiar with the above-identified application for United States Letters Patent and the Office Action thereto, mailed September 9, 1980;

THAT she tested samples of the compounds listed in Tables I-IV according to the following procedures, in her laboratory at the aforesaid Lederle Laboratories Division, Pearl River, New York;

Lymphocytic leukemia P388 test

The animals used are CD₂F₁ mice all of one sex, weighing a minimum of 18 g. and all within a 3 gram weight range. There are 5 or 6 animals per test group. The tumor transplant is by intraperitoneal injection of 0.1 ml. of dilute ascitic fluid containing 10⁶ cells of lymphocytic leukemia P388. The test compounds are administered intra-

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peritoneally on days one, 5 and 9 (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 30 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals are calculated. The positive control compound is 5-fluorouracil given as an injection at the indicated dose. The results of this test appear in Table I. The criterion for efficacy is $T/C \times 100 \geq 125\%$.

TABLE I
Lymphocytic Leukemia P388 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-dimethylaminoethyl)- amino]-5,8-dihydroxy-anthraquinone	200	7	74
	100	25 (2)*	243 (2)*
	50	24 (2)	243 (2)
	25	23 (2)	232 (2)
	12.5	18 (2)	182 (2)
	6	16	160
	3	14.5	145
	1.5	13	130
Control 5-Fluorouracil	0	10 (2)	-
	60	19 (2)	198 (2)

*In all instances, in this and the following tables, the figure in parentheses gives the number of tests run at that dose level and the figures for Median Survival Time and T/C x 100 are the average of those tests. Where no parenthetical number is given, the test was run once at that dose level.

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[(2-dimethylaminoethyl)amino]- -5,8-dihydroxy-anthraquinone	200	11 (2)	102 (2)
	100	15 (2)	142 (2)
	50	23 (5)	216 (5)
	25	21 (5)	191 (5)
	12	23 (5)	202 (5)
	6	22 (3)	196 (3)
	3	19 (3)	173 (3)
	1.5	20 (2)	156 (2)
	0.75	16	145
	0.37	15	136
	0.18	12	109
	0	12	-
Control 5-Fluorouracil	60	19 (4)	177 (4)
	20	29	187
Leuco-1,4-bis(2-morpholinoethyl- amino)-5,8-dihydroxy-anthraquinone	400	14	133
	200	13 (2)	130 (2)
	100	12 (2)	120 (2)
	50	12 (2)	115 (2)
	25	12 (2)	115 (2)
Control 5-Fluorouracil	0	10 (2)	-
	60	17	189

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis(2-morpholinoethylamino)-5,8- -dihydroxy-anthraquinone	400	11	105
	200	12 (2)	110 (2)
	100	12 (2)	115 (2)
	50	11	105
	25	11	105
Control 5-Fluorouracil	0	10 (2)	-
	60	17 (2)	174
Leuco-1,4-bis[(2-diethylaminoethyl)- amino]-5,8-dihydroxy-anthraquinone	300	20	200
	200	17 (2)	177 (2)
	100	17 (2)	169 (2)
	50	16 (2)	149 (2)
	25	13 (2)	131 (2)
	12	13 (2)	131 (2)
	0	10 (2)	-
Control 5-Fluorouracil	60	21 (2)	217 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[(2-diethylaminoethyl)amino]- -5,8-dihydroxy-anthraquinone	300	17.5	167
	200	19 (2)	188 (2)
	100	18 (2)	178 (2)
	50	16 (2)	155 (2)
	25	16 (2)	155 (2)
	12	14 (2)	134 (2)
Control 5-Fluorouracil	0	10 (2)	-
	60	19.5 (2)	195 (2)
Leuco-7,4-bis[[2-(1-pyrrolidinyl)- ethyl]amino]-5,8-dihydroxy- -anthraquinone	300	11	100
	200	20 (2)	185 (2)
	100	18 (2)	159 (2)
	50	15 (2)	136 (2)
	25	14 (2)	127 (2)
	12	14	127
Control 5-Fluorouracil	0	11 (2)	-
	60	19 (2)	173 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[[2-(1-pyrrolidinyl)ethyl]- amino]-5,8-dihydroxy-anthraquinone	100	17 (2)	150 (2)
	50	21 (2)	191 (2)
	25	19 (2)	175 (2)
	12	18 (2)	157 (2)
	6	14.5	132
	3	13.5	123
	1.5	13	118
Control 5-Fluorouracil	0	11 (2)	-
	60	20 (2)	184 (2)
1,4-Bis[(3-dimethylamino)propyl]- amino]-5,8-dihydroxy-anthraquinone	50	15.5	129
	25	15.5	125
	12	15	125
Control 5-Fluorouracil	0	12	-
	60	19.5	162

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TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-aminoethyl)amino]- -5,8-dihydroxy-anthraquinone	100	11 (3)	98 (3)
	50	15 (3)	135 (3)
	25	21 (3)	181 (3)
	12	21 (3)	189 (3)
	6	19 (2)	171 (2)
	3	18.5	168
	1.56	18	164
	0.78	15.5	141
	0.39	17	155
	0.19	18	177
Control	0	11 (3)	-
5-Fluorouracil	60	19 (3)	166 (3)
Leuco-1,4-bis(3-aminopropylamino)- -5,8-dihydroxy-anthraquinone	400	28	254
	200	19 (2)	161 (2)
	100	19 (2)	166 (2)
	50	17 (2)	144 (2)
	25	18 (2)	157 (2)
	12	16	133
Control	0	12 (2)	-
5-Fluorouracil	60	20 (2)	172 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-methylaminoethyl- amino)ethylamino]-5,8-dihydroxy- -anthraquinone	200	10 (2)	84 (2)
	100	21 (2)	189 (2)
	50	21 (2)	190 (2)
	25	17 (2)	149 (2)
	12	16	145
	6	15	136
	0	12 (2)	-
Control 5-Fluorouracil	60	18 (2)	164 (2)
Leuco-1,4-bis[2-dimethylaminopropyl- amino]-5,8-dihydroxy-anthraquinone	200	17 (3)	170 (3)
	100	15 (3)	149 (3)
	50	14 (3)	143 (3)
	25	13 (3)	129 (3)
	12	12 (3)	119 (3)
	6	11	116
	3	11	116
	1.5	11	116
	0.7	11	116
	0	10 (3)	-
Control 5-Fluorouracil	60	23 (3)	232 (3)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-(2-hydroxyethylamino)- ethylamino]-5,8-dihydroxy- -anthraquinone, dihydrochloride	25	9	82
	12	11 (3)	110 (3)
	6	15 (5)	138 (5)
	3	26 (5)	246 (5)
	1.56	28 (5)	247 (5)
	0.78	24 (5)	224 (5)
	0.39	29 (5)	226 (5)
	0.18	22 (5)	212 (5)
	0.09	22 (3)	216 (3)
	0.04	22 (2)	213 (2)
	0.02	19	173
	0	11 (5)	-
	60	20 (5)	188 (5)
Control 5-Fluorouracil			
1,4-Bis[2-(1-piperazinyl)ethylamino]- -5,8-dihydroxy-anthraquinone	200	5 (3)	51 (3)
	100	18 (3)	180 (3)
	50	14 (3)	142 (3)
	25	15 (3)	148 (3)
	12	14 (3)	142 (3)
	6	15	143
	3	11	105
	1.5	12	114
	0.7	11	105
	0	10 (3)	-
	60	21 (3)	202 (3)
Control 5-Fluorouracil			

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-(methylamino)ethylamino]- -5,8-dihydroxy-anthraquinone, dihydrochloride	25	8 (2)	75 (2)
	12	14 (2)	126 (2)
	6	22 (2)	204 (2)
	3	21 (2)	196 (2)
	1.5	22 (2)	207 (2)
	0.78	18.5	176
	0.39	19.5	186
	0.19	18.5	176
	0.09	18	171
	0.04	17	162
Control	0	11 (2)	-
5-Fluorouracil	60	9 (2)	172 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-hydroxyethyl- amino)ethylamino]-5,8-dihydroxy- -anthraquinone	200	5	45
	100	5	45
	50	10 (2)	87 (2)
	25	12 (6)	113 (6)
	12	23 (6)	210 (6)
	6	27 (5)	248 (5)
	3	27 (5)	253 (5)
	1.5	26 (5)	244 (5)
	0.78	23 (4)	219 (4)
	0.39	20 (4)	188 (4)
	0.19	20 (4)	186 (4)
	0.09	20 (3)	185 (3)
	0.04	19 (3)	169 (3)
	0	11 (6)	-
Control 5-Fluorouracil	60	19 (6)	174 (6)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(4-aminobutylamino)-5,8-dihydroxy-anthraquinone	400	20	190
	300	18	171
	200	16 (2)	143 (2)
	100	14 (2)	125 (2)
	50	14	117
	25	13	108
	12	13	108
	0	11 (2)	-
Control 5-Fluorouracil	60	19 (2)	176 (2)
Leuco-1,4-bis[2-(methylamino)ethyl-amin]-5,8-dihydroxy-anthraquinone	50	13 (2)	111 (2)
	25	20 (2)	170 (2)
	12	19 (2)	162 (2)
	6	19 (2)	167 (2)
	3	16 (2)	139 (2)
	1.56	14 (2)	122 (2)
	0.39	15	125
	0.19	15	125
	0	12 (2)	-
	60	19 (2)	166 (2)
Control 5-Fluorouracil			

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-isopropylamino)- ethylamino]-5,8-dihydroxy-anthra- quinone	100	8	73
	50	19	173
	25	17	155
	12.5	15	136
Control 5-Fluorouracil	0	11	-
	60	20.5	186
1,4-Bis[2-(2-aminoethylamino)ethyl- amino]-5,8-dihydroxy-anthraquinone	200	17	162
	100	16	152
	50	14	133
	25	13	124
Control 5-Fluorouracil	0	10.5	...
	60	17	162

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-[2-[di(β-hydroxyethyl)- amino]ethylamino]-5,8-dihydroxy- -anthraquinone	400	24 (2)	226 (2)
	300	16.5	165
	200	20 (2)	186 (2)
	100	18 (2)	167 (2)
	50	17 (2)	162 (2)
	25	16 (2)	150 (2)
	12	15 (2)	140 (2)
	6	14 (2)	128 (2)
Control 5-Fluorouracil	3	14.5	132
	0	11 (2)	-
	60	24	218
	40	18	180
1,4-Bis[2-(2-hydroxy 1-propylamino)- ethylamino]-5,8-dihydroxy-anthra- quinone, dihydrochloride	25	11 (2)	99 (2)
	12	27 (2)	257 (2)
	6	27 (2)	252 (2)
	3	24 (2)	199 (2)
	1.56	20 (2)	191 (2)
	0.78	21 (2)	198 (2)
	0.39	18 (2)	170 (2)
	0.19	19	173
	0.10	18.5	168
	0	11 (2)	-
Control 5-Fluorouracil	60	24	218
	40	18	180

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-[2-(1-morpholino)ethyl- amino]ethylamino]-5,8-dihydroxy- -anthraquinone tetrahydrochloride	200	18 (2)	166 (2)
	100	20 (2)	187 (2)
	50	18 (2)	172 (2)
	25	19 (2)	184 (2)
	12	16 (2)	148 (2)
	6	16 (2)	148 (2)
	3	14 (2)	136 (2)
	1.56	15	136
	0.78	13.5	123
	0.39	12	109
	Control	11 (2)	-
	5-Fluorouracil	24	218
	40	18	180
1,4-Bis[2-(3-hydroxy-1-propylamino)- ethylamino]-5,8-dihydroxy-anthra- quinone, dihydrochloride	25	10 (2)	99 (2)
	12	21 (2)	252 (2)
	6	24 (2)	226 (2)
	3	22 (2)	211 (2)
	1.56	22 (2)	210 (2)
	0.78	20 (2)	190 (2)
	0.39	20.5	205
	0.19	19.5	195
	Control	11 (2)	-
	5-Fluorouracil	20	200
	40	18	164

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(3-hydroxy-1-propyl- amino)ethylamino]-5,8-dihydroxy- -anthraquinone	200	12 (2)	114 (2)
	100	34 (2)	359 (2)
	50	32 (2)	291 (2)
	25	24 (2)	214 (2)
	12	21 (2)	181 (2)
	6	19 (2)	173 (2)
	3	20 (2)	182 (2)
	1.56	19 (2)	171 (2)
	0.78	18 (2)	166 (2)
	0.39	17	155
	0.19	16	145
Control 5 Fluorouracil	0	11 (2)	-
	60	19	173
	40	17	155

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (percent)
1,4-Bis[2-[di(β-hydroxyethyl)amino]- ethylamino]-5,8-dihydroxy-anthra- quinone, dihydrochloride	600	9	90
	500	29	290
	400	30	300
	300	30	300
	200	29 (3)	291 (3)
	100	22 (3)	223 (3)
	50	22 (3)	217 (3)
	25	22 (3)	217 (3)
	12	20 (2)	213 (2)
	6	19 (2)	200 (2)
	3	19 (2)	200 (2)
	1.56	17 (2)	177 (2)
	0.78	15 (2)	156 (2)
	0.39	12.5	125
	0.19	12	120
	0.10	11.5	115
Control	0	10 (3)	-
5-Fluorouracil	60	21 (3)	208 (3)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[3-(2-hydroxyethyl- amino)-1-propylamino]-5,8-dihydroxy- -anthraquinone	200	19 (2)	177 (2)
	100	27 (2)	254 (2)
	50	24 (2)	221 (2)
	25	20 (2)	189 (2)
	12	19 (2)	177 (2)
	6	19 (2)	173 (2)
	3	17 (2)	156 (2)
	1.56	16	152
	0.78	14	133
	0	11 (2)	-
	40	18 (2)	170 (2)
Control 5-Fluorouracil			
Leuco-1,4-bis[2-(2-hydroxy-1-propyl- amino)ethylamino]-5,8-dihydroxy- -anthraquinone	200	11 (2)	103 (2)
	100	27 (2)	254 (2)
	50	26 (2)	240 (2)
	25	22 (2)	205 (2)
	12	23 (2)	212 (2)
	6	19 (2)	177 (2)
	3	20	190
	1.56	18.5	176
	0.78	18.5	176
	0	11 (2)	-
	40	18 (2)	170 (2)
Control 5-Fluorouracil			

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[3-(2-hydroxyethylamino)-1-propylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	200	4	38
	100	12 (2)	110 (2)
	50	31 (2)	284 (2)
	25	25 (2)	235 (2)
	12	22 (2)	203 (2)
	6	18 (2)	170 (2)
	3	19 (2)	172 (2)
	1.56	16 (2)	149 (2)
	0.78	15 (2)	140 (2)
	0	11 (2)	-
Control 5-Fluorouracil	40	18 (2)	170 (2)
1,4-Bis[2-(1-aziridino)ethylamino]-5,8-dihydroxy-anthraquinone	200	26.5	265
	100	28.5	285
	50	21.5	215
	25	20	200
	12	20.5	205
	6	18.5	185
	3	19.5	195
	1.56	17	170
	0.78	14	140
	0	11	-
Control 5-Fluorouracil	60	20.5	205

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-(2-methylaminoethylamino)- ethylamino]-5,8-dihydroxy-anthra- quinone, tetrahydrochloride	100	22	220
	50	22	220
	25	19.5	195
	12	17	170
	6	16	160
	3	13.5	135
Control 5-Fluorouracil	1.56	13	130
	0	10	-
1,4-Bis(2-Aminoethylamino)-5,8- -dihydroxy-anthraquinone, dihydro- chloride	40	16	160
	200	3	27
	100	3	27
	50	3	27
	25	6	55
	12	7	64 (3)
	6	14 (3)	123 (3)
	3	27 (2)	245 (2)
	1.56	20 (2)	180 (2)
	0.78	23 (2)	222 (2)
	0.39	23 (2)	209 (2)
	0.19	21 (2)	193 (2)
	0.09	19.5	177
	0	11 (3)	-
Control 5-Fluorouracil	60	20 (3)	182 (3)

Lymphocytic leukemia P388 test

The procedure used is the same as for the previously described test for lymphocytic leukemia P388 except that the test compounds are administered orally at various doses rather than intraperitoneally. The results of this test with typical compounds of the present invention appear in Table II. The criterion for efficacy is $T/C \times 100 \geq 125\%$.

Table II

Lymphocytic Leukemia P388 Test (Oral Drug Administration)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-dimethylaminoethyl)amino]-5,8-dihydroxy- -anthraquinone	50 25 12	16 13.5 12.5	160 135 125
Control	0	10	-
5-Fluorouracil (administered intraperitoneally)	60	19	190
1,4-Bis[(2-dimethylaminoethyl)amino]-5,8-dihydroxy- -anthraquinone	200 100 50 25 12 6 3	6 8 10 11 17 (2) 16 15	60 70 100 110 155 (2) 139 130
Control	0	11 (2)	-
5-Fluorouracil (administered intraperitoneally)	60	19 (2)	177 (2)

Melanotic Melanoma B16

The animals used are BD_{2F_1} mice, all of the same sex, weighing a minimum of 17 g. and all within a 3 g. weight range. There are normally 10 animals per test group. A one-gram portion of melanotic melanoma B16 tumor is homogenized in 10 ml. of cold balanced salt solution and a 0.5 ml. aliquot of the homogenate is implanted intraperitoneally into each of the test mice. The test compounds are administered intraperitoneally on days one, five and nine or one through nine (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 60 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals are calculated. The positive control compound is 5-fluorouracil given as a 20 or 60 mg./kg. injection. The results of this test appear in Table III. The criterion for efficacy is $T/C \times 100 \geq 125\%$.

Table III

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis [(2-dimethylaminoethyl) amino]-5,8-dihydroxy- -anthraquinone	50	22	147
	25	25 (2)	156 (2)
	12	23 (2)	143 (2)
	6	21.5 (2)	137 (2)
	3	21 (2)	135 (2)
Control 5-Fluorouracil	0	16 (2)	-
	20	25 (2)	161 (2)
1,4-Bis [(2-dimethylaminoethyl) amino]-5,8-dihydroxy- -anthraquinone	25	24.5	136
	12	28.5	158
	6	27	150
	3	25.5	142
	0	18	-
Control 5-Fluorouracil	20	26	144

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[[2-(1-pyrrolidinyl)ethyl]amino]-5,8-dihydroxy- -anthraquinone	100	11	73
	50	22.5	150
	25	21	140
	12	18	120
	6	19.5	130
Control	0	15	-
5-Fluorouracil	20	25.5	170
1,4-bis[[2-(1-pyrrolidinyl)ethyl]amino]-5,8-dihydroxy- -anthraquinone	25	24.5	158
	12	26.5	171
	6	22	142
	3	20	129
	1.5	15.5	100
Control	0	15.5	-
5-Fluorouracil	20	29.5	190

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[(3-dimethylaminopropyl)amino]-5,8-dihydroxy- -anthraquinone	25	20	125
Control	0	16	-
5-Fluorouracil	20	26.5	166
Leuco-1,4-bis[(2-aminoethyl)amino]-5,8-dihydroxy- -anthraquinone	12	15	88
	6	38.5	226
	3	54.5	321
	1.5	55	324
	0.75	45	265
	0.39	45	265
	0.19	40.5	238
Control	0	17	-
5-Fluorouracil	20	22	165

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(3-aminopropylamino)-5,8-dihydroxy-anthra- quinone	100 50 25 12	37 31 24 26	200 168 130 141
Control 5-Fluorouracil	0 20	18.5 29	- 157
Leuco-1,4-bis[2-(2-methylaminoethyl)amino]-5,8-dihydroxy- -anthraquinone	50 25 12 6	12.5 35 30.5 28.5	73 206 232 168
Control 5-Fluorouracil	0 20	17 30	- 176

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-(1-piperazinyl)ethylamino]-5,8-dihydroxy-anthra- quinone	50 25 12 6 3	34.5 30.5 26 22 20.5	203 179 153 129 121
Control 5-Fluorouracil	0 20	17 30	- 176
1,4-Bis[2-(2-aminoethylamino)ethylamino]-5,8-dihydroxy- -anthraquinone	50 25 12 6	24 22.5 22 20	150 141 138 125
Control 5-Fluorouracil	0 20	16 27	- 169

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(2-dimethylaminopropylamino)-5,8-dihydroxy- -anthraquinone	100	21	124
	50	28.5	168
	25	24.5	144
	12	20.5	121
	6	19.5	115
	0	17	-
Control 5-Fluorouracil	20	30	176
1,4-Bis[2-(2-hydroxyethylamino)ethylamino]-5,8-dihydroxy- -anthraquinone, dihydrochloride	12	11	73
	6	16 (5)	96 (5)
	3	39 (5)	235 (5)
	2	24	170
	1.5	35 (8)	219 (8)
	1	60	300
	0.78	44 (8)	238 (8)
	0.39	39 (8)	212 (8)
	0.25	34.5	172
	0.19	33 (7)	170 (7)
	0.12	32.5	162
	0.09	19 (3)	147 (3)
	0.06	30 (2)	142 (2)
	0.04	29 (3)	141 (3)
	0	19 (9)	-
Control 5-Fluorouracil	60	24 (4)	144 (4)
	20	27 (5)	155 (5)

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-isopropylamino)ethylamino]-5,8-dihydroxy- -anthraquinone	50	6.5	39
	25	31	188
	12	30	182
	6	25	151
	0	16.5	-
Control 5-Fluorouracil	20	16.5	100
1,4-Bis[2-(methylamino)ethylamino]-5,8-dihydroxy-anthra- quinone, dihydrochloride	12	11.5	59
	6	18 (2)	96 (2)
	3	49 (2)	281 (2)
	1.5	40 (2)	235 (2)
	0.75	37 (2)	215 (2)
	0.39	31 (2)	182 (2)
	0.15	29.5	151
	0	17.5 (2)	-
Control 5-Fluorouracil	60 20	25 27.5	128 172

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(4-aminobutylamino)-5,8-dihydroxy-anthraquinone	100	21	124
	50	19 (2)	106 (2)
	25	18.5 (2)	106 (2)
	12	17 (2)	99 (2)
	6	17.5	97
Control	0	18 (2)	-
5-Fluorouracil	20	30 (2)	169 (2)
Leuco-1,4-bis[2-(2-hydroxyethylamino)ethylamino]-5,8-dihydroxy-anthraquinone	6	9 (2)	126 (2)
	3	21 (2)	174 (2)
	1.5	29 (2)	175 (2)
	0.75	29 (2)	139 (2)
	0.37	23 (2)	138
	0.18	23.5	-
Control	0	16.5 (2)	-
5-Fluorouracil	20	29 (2)	174 (2)

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(methylamino)ethylamino]-5,8-dihydroxy- -anthraquinone	50	5	32
	25	5.5	35
	12	29 (2)	187 (2)
	6	33 (2)	208 (2)
	3	31	194
	1.5	36	225
	0.7	27.5	172
Control 5-Fluorouracil	0	16 (2)	-
	20	28 (2)	180 (2)

Ridgway Osteogenic Sarcoma

The animals used are AKD₂F₁/J mice, all of the same sex, weighing a minimum of 17 g. and all within a three-gram weight range. There are normally 8 animals per test group. The tumor is administered subcutaneously by trocar as five 2 mm. fragments per mouse. The test compounds are administered intraperitoneally every 4 days for a total of 6 inoculations beginning on day 15 (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 90 days. The regression of tumors is recorded in all test animals. Table IV gives the result of this test in terms of the percentage of animals showing tumor regression.

Table IV

Ridgway Osteogenic Sarcoma

Compound	Dose (mg./kg.)	1 Day Before Therapy		7 Days After Therapy Stopped				63 Days After Therapy Stopped	
		No. Mice Per Group	Tumor (mm.)	No. Without Tumors/No. Survivors	Tumor (mm.)	% Inhibition Tumor Growth	% Showing 50% Tumor Regression	Median Survival (Days)	T/C (Percent)
Placebo	-	8	64	0/5	1189		0	44.5	
1,4-Bis[(2- -dimethyl- aminoethyl)- amino]-5,8- -dihydroxy- -anthraqui- none	100	7	77	2/5	52	96	28	48	108
	50	8	68	2/6	263	78	25	92.5	208
	25	8	82	0/8	653	41	0	78	175
	12	7	84	0/3	470	61	0	37	83
	6	7	83	0/6	960	19	0	54.5	129
Methotrexate	25	3	51	1/6	546	54	12	52.5	118
	12	8	52	0/5	916	23	0	49	110
	6	8	54	0/4	758	36	0	46	103
Vincristine	1.5	8	42	4/4	0	100	100	68	153
	1.0	6	99	6/6	0	100	100	85	191
	0.5	7	94	4/7	77	93	57	83	186

THAT the foregoing results show the efficacy of the named compounds in inhibiting the growth of transplanted mouse tumors;

Further deponent sayeth not.

Roslyn E. Wallace
ROSLYN E. WALLACE

Sworn to and subscribed before me this 15th day of

December 1980.

Kimberly E. Miner
NOTARY PUBLIC

KIMBERLY E. MINER
Notary Public, State of N. Y.
No. 41-2724350
Residing in Rockland County
Commission Expires March 30, 1981